

Age determination procedures for benthic fish in Spanish Institute of Oceanography (IEO)

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Abstract

This handbook presents a summary of the age estimation procedures used in the Spanish Institute of Oceanography (IEO) for some of the main commercial benthic species of fish for the Spanish fleet: megrim (*Lepidorhombus whiffiagonis*), four spot megrim (*Lepidorhombus boscii*), white anglerfish (*Lophius piscatorius*), black anglerfish (*Lophius budegassa*). It provides information about the sampling program, the morphology of hard parts (otoliths and illicia), their extraction, preparation, and the age estimation criteria. A summary of information related to the accuracy, validation and corroboration of age of each species is also presented, as well as that related to the precision, quality control and verification of age.

Keywords: age, growth, hard part, calcified structure, otolith, illicia, *Lepidorhombus whiffiagonis*, *Lepidorhombus boscii*, *Lophius piscatorius*, *Lophius budegassa*.

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INTRODUCTION

Since 1990s, the age of commercial benthic fish is been determined on the basis of hard parts, (*illicia* for both anglerfish species, and *sagittae* otoliths for megrim and four spot megrim), within the Spanish Institute of Oceanography/Instituto Español de Oceanografía (IEO).

For monitoring fish populations, biological samplings are performed throughout the year. These samplings are carried out once a month or quarter, depending on the stock and species. The samples come from the fish market (fish landed from the commercial fleet) or from bottom trawl research surveys (“Demersales” and “Porcupine”). Some years, depending on the sampling program or research project, the hard parts have also been taken by observers on board of commercial vessels.

Annual age estimates are mainly used for stock assessment, but also for studies on age and growth. Daily growth studies in both anglerfish species have been also recently begun.

Currently four age readers carry out the age estimation of the Atlantic stocks of the aforementioned benthic fish in IEO. These age readers participate in the periodic international age estimation exchanges and workshops.

Hard parts are sampled by the IEO within the EU data collection framework (DCF). Detailed manuals of each processing step (sampling, preparation of the hard part, age estimation criterion, etc.) are available in Spanish.

1 MEGRIM (*LEPIDORHOMBUS WHIFFIAGONIS*)



Fig. 1.1. Megrime (*L. whiffiagonis*)

Species identification

By Whitehead *et al.* (1986):

- Flatfish with eyes on the left side and preopercular margin free.
- Large mouth with prominent lower jaw.
- Bases of both pelvic fins elongate. Dorsal fin origin closer to tip of snout than to anterior edge of eye.
- Lateral line forms a distinct curve above pectoral fin.
- The main distinguishing morphological characteristics of this species with its congener are the dorsal and anal fins with indefinite darker spots posteriorly (Fig. 1.1) and the dorsal fin with 85-94 rays.

Otolith morphology

Shape: oval-elliptical, with sinuous dorsal margin. *Sulcus acusticus*: heterosulcoid, ostial, mid. *Ostium*: tubular, straight, much longer than the cauda. *Cauda*: oval-round, very short, as wide as the ostium, terminating away from the rear margin. *Anterior region*: angled; small *rostrum*, broad, round or pointed; *antirostrum* absent or poorly defined, small, wide, round or pointed, narrow; narrow *excisura* with or without a shallow notch. *Posterior region*: angled to rounded. *Circumsulcal depression* incomplete, about three quarters of the sulcus, narrow and close to the margins (Tuset *et al.*, 2008).

1.1 SAMPLING PROGRAM FOR AGE ESTIMATION

1.1.1 ANNUAL AGE

Samplings for age determination of megrim are performed in IEO from the late 80s, from both, commercial catches and research surveys. The number of otoliths collected in 2012 is shown in Table 1.1.1.1.

Table 1.1.1.1. Number of megrim (*L. whiffiagonis*) otoliths by stock collected from the commercial fleet and research surveys in 2012.

Stock	Commercial fleet	Research surveys	Total
ICES Div. VIIb-k, VIIIabd	1427	699	2126
ICES Div. VIIIC, IXa	493	396	889
Total	1920	1095	3015

1.2 OTOLITH EXTRACTION AND STORAGE

1.2.1 ANNUAL AGE

To extract the otoliths, the megrim is held and, taking its operculum as a reference, a path is followed up vertically up to its dorsum where a cut is performed with a sharp knife, sectioning its skull (Fig. 1.2.1).



Fig. 1.2.1. Cutting for the otoliths extraction of a megrim.

The section is held open, observing that the otoliths are located within the skull, in two sinuses on each side of the brain. The tips of straight metal laboratory tweezers are introduced

smoothly to extract each otolith from those spaces. The megrim otoliths are robust, and depending on the skill of the cutting, sometimes they will be more in sight; other times they will be more hidden, so they have to be searched with tweezers, to the touch (Fig. 1.2.2).



Fig. 1.2.2. Removing otoliths from a megrim.

After both otoliths have been extracted from each megrim, the accompanying organic remains are removed with tweezers, finely, for not to break them. Both otoliths from each megrim are stored in a paper envelope (or in a vial), in which the data of the sampled specimen have been previously written down (Fig. 1.2.3).



Fig. 1.2.3. Clean pair of megrim otoliths to keep on an envelope.

1.3 OTOLITH PREPARATION AND AGE ESTIMATION METHOD

1.3.1 ANNUAL AGE

Preparation: after the two otoliths are removed from their respective storage envelope, are included in a black plastic cavity with water, in order to highlight their growth increments and facilitate the age estimation. Years ago they were included in an aqueous solution of glycerol to 40%, for a period of approximately 24 hours before reading, but this methodology was not substantially improved the age interpretation. Once estimated age, otoliths are dried and deposited back into their respective storage envelopes.

Observation: binocular microscope.

Illumination: under reflected light (using fiber optic illuminators), on a black background.

Magnification: 8-15x.

Growth increments: those translucent (hyaline) increments, clearly marked and comparatively wider than others, were considered annual and counted.

Calcified structure and reading area: the age is estimated by observing both otoliths. Of the several areas of the otolith where the age can be estimated, the anterior (*rostrum*) of the left otolith is that with higher distance from the center to its edge, being the area where the annuli are better distinguished and it is the firstly used to estimate the age. Next, the right otolith is observed in both anterior and posterior (*post-rostrum*) areas (Fig. 1.3.1.1). Some few left otoliths have broken the anterior area, due to problems in the extraction or handling, and age in that area is not easily estimated.

Age estimation criterion: the age estimation criterion here followed was basically that of Anon (1997) for *L. whiffiagonis*, being similar otoliths and criterion for both *Lepidorhombus* species. The age estimation criterion has not substantially shifted from when its age began to be estimated (eg. Conan *et al.*, 1981). For the interpretation of the otolith edge, additional information to the otolith structure is considered, as the date of capture, the spawning season (peak mainly from February to April) and the main period of seasonal increment formation (ICES, 2013). Table 1.3.1.1 shows the overall agreed scheme of Anon (1997) used for interpretation of the edge. Hyaline edge in the 2nd or 3rd quarter is unusual because it is mostly predominant from October to April (Rodríguez and Iglesias, 1985).

Table 1.3.1.1. Overall scheme used for interpretation of the otolith edge of megrim (*L. whiffiagonis*). N= number of hyaline annulus.

Edge type	Quarter 1	Quarter 2	Quarter 3	Quarter 4
Hyaline	Age = N	Age = N	Age = N-1	Age = N-1
Opaque	Age = N+1	Age = N	Age = N	Age = N

Difficulties in age interpretation: the age estimation of *L. whiffiagonis* is not especially difficult compared with that of other fish, and the annuli can be observed in many otoliths (Fig. 1.3.1.1).

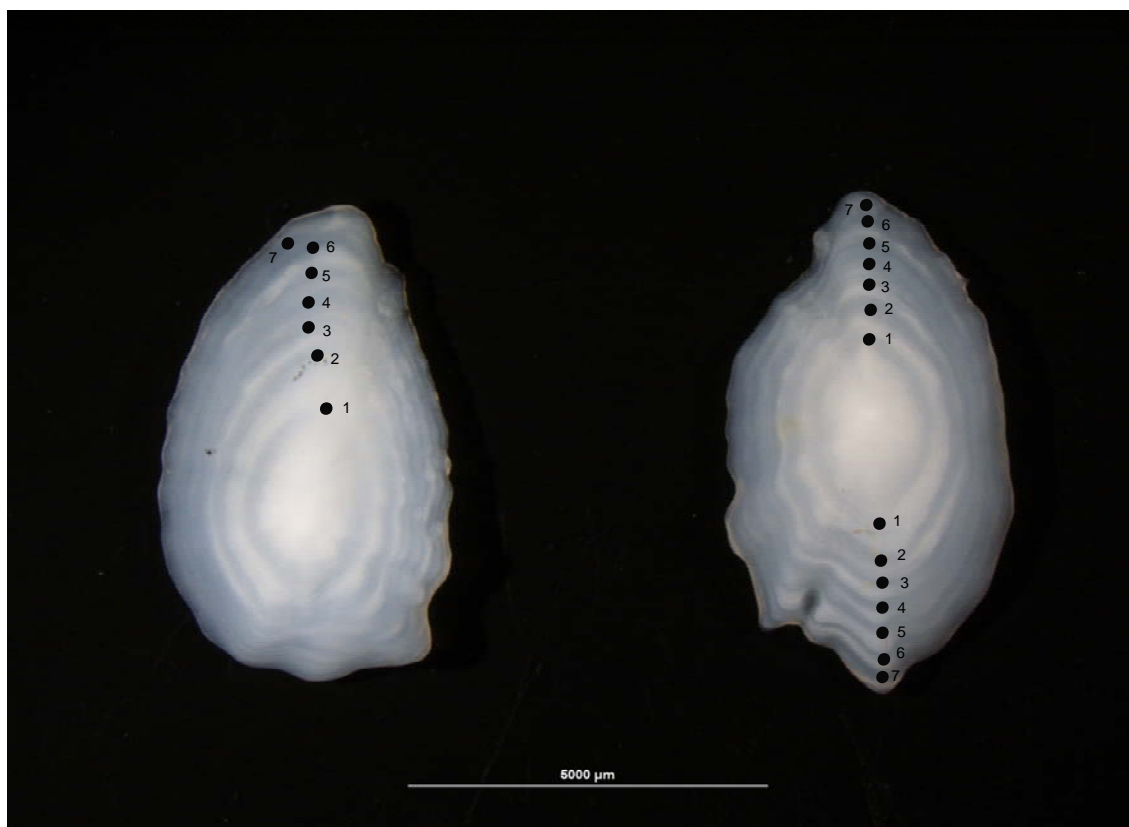


Fig. 1.3.1.1. Megrim (*L. whiffiagonis*) otoliths of 7 years of estimated age, captured in the 4th quarter. The three main reading areas are showed. Each hyaline annulus counted is marked with a spot and number.

1.4 AGE ACCURACY, VALIDATION AND CORROBORATION

1.4.1 ANNUAL AGE

Validation of an absolute age is equivalent to determining the accuracy of an age estimate. The distinction between validating the periodicity of growth increment formation and absolute age is important. Marginal increment analysis is the most commonly used of the validation methods, and it is used for validating the periodicity of growth increment formation (Campana, 2001). Edge analysis does not assign a state of completion to the marginal increment, but rather records its presence as either an opaque or translucent zone (Campana, 2001).

The periodicity of growth increment formation in *L. whiffiagonis* otoliths has been validated by edge analysis in several areas of northeast Atlantic (ICES Div. VII, VIIIabc, IXa) (Rodríguez and Iglesias, 1985; Landa and Piñeiro, 2000). The season in which the hyaline annuli are formed was determined by examination of the frequency distribution of otolith edge types throughout the year. The hyaline edge is mostly predominant from October (Rodríguez and Iglesias, 1985) and during the first four months of the year, while the opaque edge predominates in the rest

of the year (Rodríguez and Iglesias, 1985; Landa and Piñeiro, 2000). April is the month in which the hyaline annulus formation terminates.

Methods for age corroboration are not equivalent to those for age validation, since corroboratory methods support or are correlated with a particular method of ageing, but are not directly or logically linked (Campana, 2001). The corroboration method of tracking of strong/weak year-classes compares the interval between yearly samples and the increase in the apparent modal age of a recruitment pulse as determined through annulus counts (Campana, 2001). This also considered “indirect validation” method indicates that an age-reading method is accurate if the age composition of exceptionally good or weak year classes can be tracked over a long period of time (Panfili *et al.*, 2002).

The age estimation criterion of *L. whiffiagonis* was preliminary corroborated (or indirectly validated) by tracking year-classes abundance indices in the research surveys in the north and northwestern Iberian Peninsula (ICES Divisions VIIIc and IXa) (ICES, 1995; Sánchez *et al.*, 1998).

1.5 AGE PRECISION, VERIFICATION AND QUALITY CONTROL

1.5.1 ANNUAL AGE

Verification confirms the consistency of the age interpretation, i.e. the repeatability and/or precision of a numerical interpretation that may be independent of the age. Considerable efforts are made by international committees to standardize the age interpretations (Panfili *et al.*, 2002). ICES WKNARC-2 recommended internal (within each research institute) and external (among institutes) quality controls to confirm the consistency of the age interpretation (ICES, 2013).

The age estimation criterion of *L. whiffiagonis* used by IEO readers was externally verified in international otoliths exchanges and workshops (Anon, 1991; Dawson, 1991; Anon, 1997; Egan *et al.*, 2004; Etherton, 2011). The current IEO age readers participated in the recent otoliths exchanges and workshops, showing good values of agreement, precision and relative accuracy (Egan *et al.*, 2004; Etherton, 2011).

In addition to the estimated age, the quality (or credibility) of each age estimation is also assigned according to the "3 point grading system" recommended WKNARC-2 (ICES, 2013). Three possible results of age quality (AQ) are distinguished:

AQ1. Otoliths easy to age whose estimated age was not doubtful at first reading. The estimated age was considered as the final age for that individual;

AQ2. Otoliths difficult to read, whose estimated age in 1st reading was doubtful and were again examined. If the estimated 2nd age was the same as in the 1st, that age was assigned as the final age of the individual. If doubts between the two ages still remained, the otolith was read a 3rd time, being assigned the most frequent age of three values, or leaving the age with

two values (eg 5/4). For the preparation of age-length keys, those otoliths with doubts between two ages, were assigned as belonging to the more confident age (the value firstly located, eg. 5 for age 5/4);

AQ3. Otoliths practically unreadable or very difficult to age, with doubts among three or more possible ages. Those otoliths were excluded from further analysis.

2 FOUR SPOT MEGRIM (LEPIDORHOMBUS BOSCI)



Fig. 2.1. Four spot megrim (*L. boscii*), showing the four spots in the dorsal and ventral fins.

Species identification

By Whitehead *et al.* (1986):

The main morphological characteristics of this species are similar to those of its congener megrim, differing morphologically from it mainly by the two distinct posterior on both dorsal and anal fin (Fig. 2.1) and the dorsal fin with 79-86 rays.

Otolith morphology

Shape: oval-elliptical. *Sulcus acusticus*: heterosulcoidal, ostial, straight, medium. *Ostium*: funnel-shaped, much longer than the cauda. *Cauda*: rounded-oval, very short, as wide as the ostium, terminating away from the rear margin. *Anterior region*: round-pointed; *rostrum* broad, rounded to pointed; *antirostrum* absent or poorly defined, short, broad, round or pointed; narrow *excisura* with or without a shallow or acute notch. *Posterior region*: angled to rounded-oblique on the back. *Circumsulcal depression*: incomplete, around the back half of the sulcus, close to the sulcus (Tuset *et al.*, 2008).

2.1 SAMPLING PROGRAM FOR AGE ESTIMATION

2.1.1 ANNUAL AGE

Samplings for age determination of four spot megrim are performed in IEO since the late 80s, from both, commercial catches and research surveys. The number of otoliths collected in 2012 is shown in Table 2.1.1.1.

Table 2.1.1.1. Number of four spot megrim (*L. boscii*) otoliths collected by stock from the commercial fleet and research surveys in 2012.

Stock	Commercial fleet	Research surveys	Total
ICES Div. VIIIc, IXa	257	709	966

2.2 OTOLITH EXTRACTION AND STORAGE

2.2.1 ANNUAL AGE

The procedure for extracting both otoliths is the same as that used for the megrim (*L. whiffiagonis*) (section 1).

2.3 OTOLITH PREPARATION AND AGE ESTIMATION METHOD

2.3.1 ANNUAL AGE

The preparation method is the same as that used for the megrim (*L. whiffiagonis*) (section 1).

The observation methodologies and age estimation criterion here followed was basically that of Anon (1997) for *L. whiffiagonis*, being similar otoliths and criterion for both *Lepidorhombus* species. The age estimation criterion has not substantially shifted from when its age began to be estimated (eg. Fuertes, 1978). The age estimation of *L. boscii* is not especially difficult compared with that of other fish, and the annuli can be observed in many otoliths (Fig. 2.3.1.1).

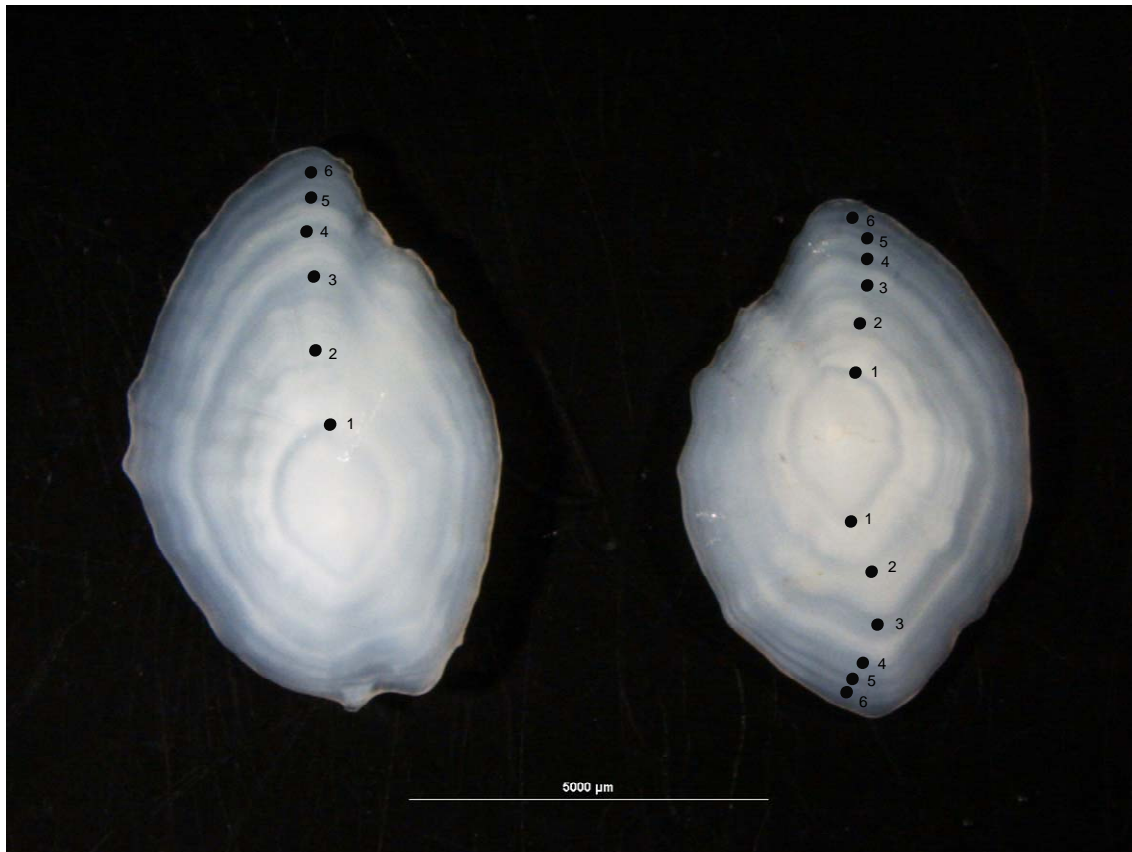


Fig. 2.3.1.1. Four spot megrim (*L. boscii*) otoliths of 6 years of estimated age, captured in the 4th quarter. The three main reading areas are showed. Each hyaline annulus counted is marked with a spot and number.

2.4 AGE ACCURACY, VALIDATION AND CORROBORATION

2.4.1 ANNUAL AGE

The periodicity of growth increment formation in *L. boscii* otoliths has been validated by edge analysis in northwestern Iberian Peninsula (ICES Div. VIIIc, IXa) (Fuentes, 1978). The season in which the hyaline annuli are formed was determined by examination of the frequency distribution of otolith edge types throughout the year. The hyaline edge is mostly predominant from November to April, while the opaque edge predominates in the rest of the year. April is the month in which the hyaline annulus formation terminates (Fuentes, 1978).

The age estimation criterion of *L. boscii* was preliminary corroborated (or indirectly validated) by tracking year-classes abundance indices in the research surveys in the north and northwestern Iberian Peninsula (ICES Divisions VIIIc and IXa) (ICES, 1995; Sánchez *et al.*, 1998).

2.5 AGE PRECISION, VERIFICATION AND QUALITY CONTROL

2.5.1 ANNUAL AGE

The age estimation criterion is similar for both *Lepidorhombus* species. Although international otoliths exchanges and workshops were not held for *L. boscii*, the current IEO age readers participated in the recent ones of *L. whiffiagonis* (section 1), showing good values of agreement, precision and relative accuracy (Egan *et al.*, 2004; Etherton, 2011).

In addition to the estimated age, the quality (or credibility) of each age estimation is also assigned according to the "3 point grading system" recommended by WKNARC-2 (ICES, 2013), distinguishing 3 possible results of age quality (AQ) as explained for *L. whiffiagonis* (section 1).

3 WHITE ANGLERFISH (LOPHIUS PISCATORIOUS)

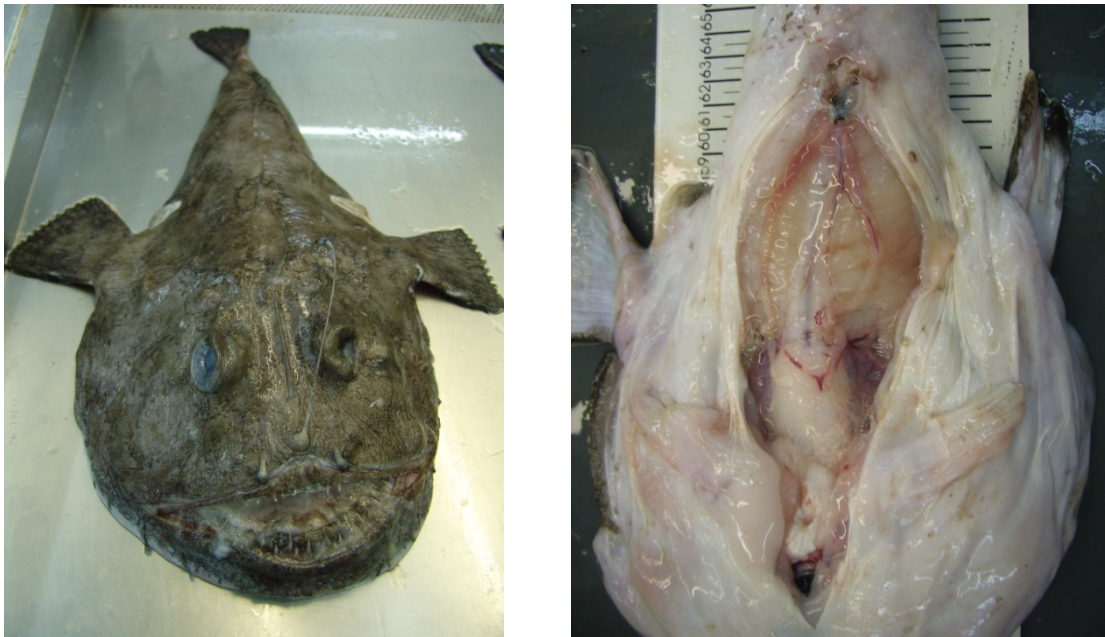


Fig. 3.1. Dorsal and ventral view of *L. piscatorius*, showing the white peritoneum.

Species identification

By Bauchot and Pras (1987):

- Dorso-ventrally depressed and symmetric body.
- Scaleless and slimy skin, brown back.
- Head disproportionately large, noticeably wider than the body, very small eyes and slit mouth, with a slightly protruding jaw and two series of strong hooked teeth.
- Wide gill cavity and small spines on maxilla and head.
- Pectoral fins with rectangular shape.
- First ray of the first dorsal fin (*illicium*) ends in a skin lobe.
- The main distinguishing morphological characteristics of this species with its congener are the white peritoneum (Fig. 3.1) and the second dorsal fin with 11-12 rays.

***Illicium* morphology**

The first ray of the first dorsal fin (*illicium*) (Fig. 3.2) is thin, flexible, very mobile and larger than the following rays, ending in a skin lobe, wiggled as bait to lure their prey stalking (Bauchot and Pras, 1987). In the first international workshop of age estimation of anglerfish (Anon, 1991), the *illicium* was considered as a better skeletal structure for age estimation, because better results were obtained than using otoliths.



Fig. 3.2. *Illicium* of anglerfish after cutting.

3.1 SAMPLING PROGRAM FOR AGE ESTIMATION

3.1.1 ANNUAL AGE

Routine samplings for age determination of *L. piscatorius* began to be performed in IEO since the mid 90s, from both, commercial catches and research surveys. The number of *illicia* collected in 2012 is shown in Table 3.1.1.1.

Table 3.1.1.1. Number of *L. piscatorius illicia* collected from the commercial fleet and research surveys in 2012.

Stock	Commercial fleet	Research surveys	Total
ICES Div. VIIb-k, VIIIabd	408	235	643
ICES Div. VIIlc, IXa	925	151	1076
Total	1333	386	1719

3.1.2 DAILY AGE

Lapilli otoliths from juvenile *L. piscatorius* caught in the routine research surveys in 2010 and 2012, were collected for daily growth studies and otolith microstructure analysis (Table 3.1.2.1).

Table 3.1.2.1. Number of *L. piscatorius lapilli* otoliths collected for daily growth studies from research surveys during 2010 and 2012 in ICES Div. VIIlc, IXa.

Year	Research surveys
2010	134
2012	55
Total	189

3.2 ILLICIA/OTOLITH EXTRACTION AND STORAGE

3.2.1 ANNUAL AGE

The *illicium* is cut at its base, lifting its apical end to facilitate cutting (Fig. 3.2.1.1). The cut *illicium* of each anglerfish is stored in an envelope, in which the data of the sampled specimen are recorded. Only the bottom of *illicium* is enough to be placed it in the envelope (Fig. 3.2.1.2).



Fig. 3.2.1.1. Cutting an *illicium* of anglerfish at its base.



Fig. 3.2.1.2. *Illicium* section to keep on an envelope.

3.2.2 DAILY AGE

To extract the *lapilli* otoliths, a dorso-ventral cut (parallel to the margin of the orbit) is made using a sharp knife, thus exposing the brain which is carefully extracted (Fig. 3.2.2.1). Through a careful observation, the semicircular canals are located along the lateral walls of the brain cavity, and the *lapilli* (Fig. 3.2.2.2) are found at the confluence of the canals (Secor *et al.*, 1992). *Lapilli* are removed using tweezers, cleaned and stored in black plastic plates labeled with the appropriate sample information.



Fig. 3.2.2.1. Macroscopic dissection technique used on juvenile anglerfish to extract otoliths.

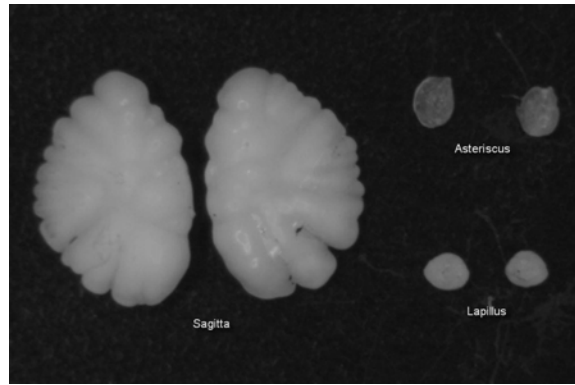


Fig. 3.2.2.2. The three pairs of anglerfish otoliths. The *lapilli* otoliths are used for daily increment analysis.

3.3 ILLICIA/OTOLITH PREPARATION AND AGE ESTIMATION METHOD

3.3.1 ANNUAL AGE

The traditional methodology of *illicia* mounting in resin plates was originally described by Dupouy *et al.* (1986) and, after several European age estimation workshops of anglerfish (Anon, 1997; Anon, 1999; Landa *et al.*, 2002), it was standardized and was included in an age estimation guide for anglerfish (Duarte *et al.*, 2002). That methodology was used in most of the growth studies using *illicia* (Duarte *et al.*, 1997; Quincoces *et al.*, 1998a; Landa *et al.*, 2001; Ofstad and Laurenson, 2007). However, several modifications in the traditional methodology of Dupouy *et al.* (1986) have been recently carried out for *illicia* preparation, observation and age interpretation (Landa *et al.*, 2013). Those methodological modifications have been performed to allow a more clear observation of the growth pattern, showing mainly the most apparent growth marks, in order to allow the distinction of the annuli:

Section thickness. The thinner cuts drove to the observation of more supposed annual increments, and vice versa using thicker cuts. The use of transverse sections ~0.50–0.55 mm thick allows the observation of the clearest marked increments, probably those that are annual. However, the use of sections thinner than 0.5 mm (~0.4 mm) produced the observation and count of some false annual increments as annuli (Landa *et al.*, 2013).

Magnification. Both the use of a profile projector 50 × (as used initially by Dupouy *et al.*, 1986), or the use of a microscope at 40x, allow a better observation of the annuli. However, the use of higher magnification (100 x), which was the standard observation methodology used by Duarte *et al.* (2002) and subsequent studies, involves observation and counting as annuli, of some false annual increments (Landa *et al.*, 2013).

Age interpretation in *illicia* consists of identifying dark and light annual increments; although for age estimation only the dark annuli are counted (Fig. 3.3.1.1). The annuli in some *illicia* are

clearly visible because they are well defined, but the increments appear doubled in others, which makes age estimation difficult.

The age estimation guide of anglerfish (Duarte *et al.*, 2002) also included some characteristics inherent to the age interpretation using *illicia* that must be considered:

It is important to adjust and play with the light and focus of the microscope, to identify an overall pattern of growth. Unlike otoliths, where the annuli widths tend to decrease as you approach the edge, in *illicia*, annuli remain a similar width apart throughout the section. Annuli close to the edge may even be wider apart than those closer to the nucleus (Duarte *et al.*, 2002).

Annuli in *illicia* differ in composition. As a result, the surface appears rippled, alternating between high and low ridges. The differences in these levels relate directly to the dark and light rings. This characteristic is very apparent from research carried out using scanning electron microscopy (Duarte *et al.*, 2002).

Annuli may not be visible in all the axes of the section. Defined annuli, which are clearly visible in one part of a section may be less defined or even appear to double in another part of the section. The counting should be based upon the area where good contrast between annuli exists (Duarte *et al.*, 2002).

The next step in the age estimation process is to identify the position of the first annulus, and to confirm it by measuring its diameter. The first well-marked growth increment observed is considered to be a consequence of a change in the life cycle (from planktonic to benthic living), and is therefore designated as the benthic growth increment. Although the next growth increment has been traditionally considered to be the first annulus, following the age estimation guide (Duarte *et al.*, 2002), the study of Wright *et al.* (2002) based on micro-increment analysis of *L. piscatorius*, concluded that growth increment (that oval shaped in Fig. 3.3.1.1) should not be considered as annulus. The following growth increment will be considered as a real annulus and its diameter tends to be 300-380 µm (Fig. 3.3.1.1) (Landa *et al.*, 2013), while the named benthic growth increment (not annual) tends to be 160-220 µm. There is not validation information on the diameters of the first growth increments for *L. budegassa*.

To identify the outer annulus it is very important to look at the *illicium* edge. For this it is essential to know the quarter (or month) in which the sample was taken. This will determine whether or not the annulus at the edge is to be counted in the age reading process. If the outer annulus is not visible in the whole *illicium*, this may be because the section has not been cut perpendicularly. When a dark annulus appears at the edge in Q1, it should be counted and included in the age reading. If a similar annulus appears in Q4 it should not be counted or included in the age reading.

It is recommended to read *illicia* of similar length group fish together, and also to begin with the clearest *illicia* sections. This is a good exercise to help train the eye in identifying the typical growth pattern of *illicia*. Because the first annuli in younger fish are often difficult to define, it

is easier to begin reading the *illicia* from the middle of the fish length range to establish the growth pattern of these first annuli (Duarte *et al.*, 2002).

The fish length can be a useful piece of information in the age estimation of its *illicium*. It is recommended to do a first reading and afterwards to check that the age reading lies within the possible mean fish length range at that age (Duarte *et al.*, 2002).

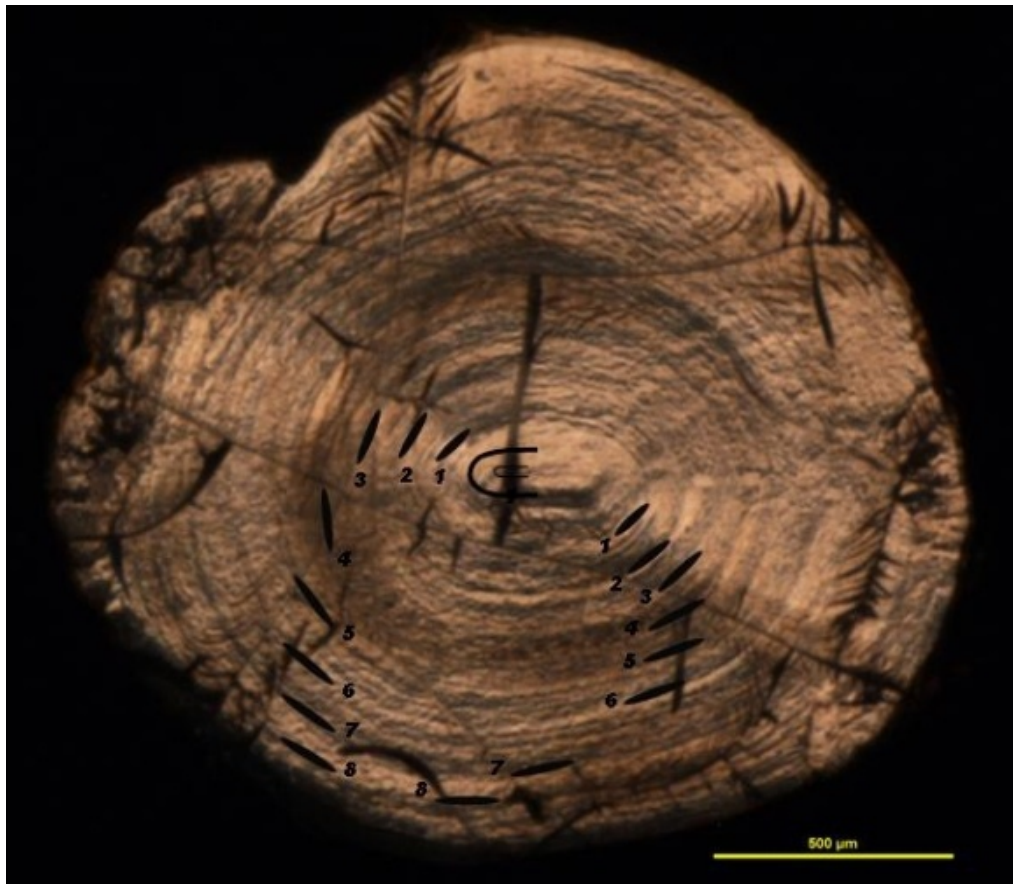


Fig. 3.3.1.1. *Illicium* of *L. piscatorius* of 89 cm and estimated 8 years old. The annuli are marked with numbers, and the two structures marked in the central area, lineal and oval in shape, are both considered checks (false annual increment) (Landa *et al.*, 2013).

Difficulties in age interpretation: the age estimation of anglerfish is not easy, mainly because annuli appear doubled or are not well defined in some *illicia* sections and false annual increments may also occur (Duarte *et al.*, 2002). As an example we can see that within the *illicia* exchange of *L. piscatorius* in 2011, the “medium” credibility level was the most frequent for most readers (50%). The “high” and “low” credibility levels were estimated in a similar proportion (around 21-25%) (Landa, 2012).

Doubts in age estimation of *illicia* of intermediate ages may be related to first maturation or any other unidentified life-history event, which causes changes in the growth pattern (Duarte *et al.*, 2002).

The age of *L. piscatorius* is not routinely estimated by the IEO age readers, although the *illicia* are being collected in the routinely samplings (section 3.1.1). Solid research steps are being taken to improve the knowledge on the real annual growth pattern of this species using CS (section 3.4.1).

3.3.2 DAILY AGE

The *lapilli* otoliths are used to examine daily increments, since they appear more clearly defined in these pair of otoliths. The *lapilli* otoliths of *L. piscatorius* are very small with a rounded shape and thickness, making them less fragile than the other two otoliths pairs.

Preparation: the *lapilli* otoliths extracted from juveniles are cut into sections by a sanding and polishing process (Secor *et al.*, 1992). Each otolith section is processed on the sagittal plane with respect to the fish.

Observation: juvenile *lapilli* otolith sections are viewed through a microscope connected to an image analyzer.

Magnification: to facilitate otolith reading a drop of immersion oil is added to the surface of each otolith and viewed at x400 magnification (and x1000 for analyzing the primordium zone) by means of a light microscope equipped with a digital camera.

Reading axes: the widths of the increments are measured along the longest radius of the *lapilli*, starting at the hatch check (Campana and Jones, 1992) to the outer edge of *lapillus* as it gives the most unambiguous sequence of growth increments.

Age estimation criteria: we assume that the first increment was formed on the day of hatching and that the other increments are laid down daily, thus the increment number is considered an indicator of the age in days. The primordium of *lapilli* consists of one or two cores followed by a few increments surrounded by well defined increments that could be most likely the hatching check. A second less evident check, the possible first feeding check, is also observed.

Interpretation difficulties: these difficulties could be explain by: 1) difficulties in the interpretation of subdaily increments; 2) to locate microstructural checks in the area surrounding the primordium; 3) unclear images, in which is difficult to interpret correctly the daily growth pattern due to under-or over-polishing, poor image acquisition. The downside of this process is that a large number of samples are rejected.

3.4 AGE ACCURACY, VALIDATION AND CORROBORATION

3.4.1 ANNUAL AGE

The age estimation from *illicia* of a decadal time-series was performed for the southern stock (Iberian Atlantic) assessment of *L. piscatorius*, using the internationally standardized age estimation criterion of Duarte *et al.* (2002). A catch-at-age by year matrix was built, but inconsistencies in cohort tracking were found (Azevedo *et al.*, 2008). Since then no age-structured model has been used for the assessment of both northern and southern stocks of the European Atlantic southern shelf of *L. piscatorius* (ICES, 2011). A production model (ASPIC, Prager, 1994) has been used for the assessment as an alternative to the age-structured models. Length-based model (CASA, Sullivan *et al.*, 1990; Dobby, 2002) including von Bertalanffy growth parameters is also being attempted for the stock assessment of *L. piscatorius*.

Growth studies alternative to the age estimates on CS of *L. piscatorius*, such as tagging-recapture (Laurenson *et al.*, 2005; Landa *et al.*, 2008), daily increment analysis (Wright *et al.*, 2002) and length frequency distributions of catches (Dupouy *et al.*, 1986; Thangstad *et al.*, 2002; Jónsson, 2007), also showed that the growth pattern estimated using the traditional age estimation criterion based on *illicia* (Duarte *et al.*, 2002) was partially underestimated and that criterion was not accurate, although it was internationally standardized and used in several age estimation anglerfish workshops (Anon 1991; Anon 1997; Anon 1999; Landa *et al.*, 2002; Duarte *et al.*, 2005). Modifications in the methodology of *illicia* preparation and in the traditional age estimation criterion have allowed a new age estimation criterion on *illicia* (Landa *et al.*, 2013). The use of this new criterion allows a better cohort tracking of the catch at age data from survey data of Porcupine Bank and is consistent with the length-frequency analyses of those data (Landa *et al.*, 2013). Another study (Ofstad *et al.*, 2013) has been recently presented on the age and growth of *L. piscatorius* in Faroese waters. These two studies presented a similar growth pattern from *illicia* and are also consistent with growth estimates from aforementioned length frequency analyses and tagging-recapture results.

Further studies on validation and corroboration of age and growth of *L. piscatorius* have been recommended (ICES, 2012).

3.4.2 DAILY AGE

The daily periodicity of micro-increment deposition has not been validated in early life stages of *L. piscatorius* by any of the validation techniques available for daily age (Campana 2001). There is one study on juvenile growth of this species in North-East Atlantic waters (Wright *et al.*, 2002).

3.5 AGE PRECISION, VERIFICATION AND QUALITY CONTROL

3.5.1 ANNUAL AGE

Verification confirms the consistency of the age interpretation, i.e. the repeatability and/or precision of a numerical interpretation that may be independent of the age. Considerable efforts are made by international committees to standardize the age interpretations (Panfili *et al.*, 2002). ICES WKNARC-2 recommended internal (within each research institute) and external (among institutes) quality controls to confirm the consistency of the age interpretation (ICES, 2013).

The first growth patterns of *L. piscatorius* in Atlantic waters estimated using hard parts (*illicia* by Dupouy *et al.*, 1986 and otoliths by Crozier, 1989) showed similarities, but with one age class of difference between them. The growth pattern is easier to distinguish in the *illicium* (Crozier, 1989; Woodroffe *et al.*, 2003) and it has become the standard structure for age estimation of *L. piscatorius* in most European countries (Landa *et al.*, 2008).

The age estimation criterion on both *Lophius* species used by IEO readers was externally verified in international *illicia* and otoliths exchanges and workshops (Anon, 1997; Anon, 1999; Landa *et al.*, 2002; Duarte *et al.*, 2005; Landa, 2012). The current age readers of IEO participated in the recent exchanges and workshops, showing good values of agreement, precision and relative accuracy for *illicia* (Duarte *et al.*, 2005; Landa, 2012). However, improving the precision in the absence of accuracy cannot, under any account, guarantee data quality (de Pontual *et al.*, 2006). And the age criteria for this species was internationally standardized and verified age of readers until 2007, did not mean that this criterion was not biased, as previously explained.

In addition to the estimated age, the quality (or credibility) of each age estimation is also assigned according to the "3 point grading system" recommended by WKNARC-2 (ICES, 2013). Three possible results of age quality (AQ) are distinguished:

AQ1. *Illicia* easy to age whose estimated age was not doubtful at first reading. The estimated age was considered as the final age for that individual;

AQ2. *Illicia* difficult to read, whose estimated age in 1st reading was doubtful and were again examined. If the estimated 2nd age was the same as in the 1st, that age was assigned as the final age of the individual. If doubts between the two ages still remained, the *illicium* was read a 3rd time, being assigned the most frequent age of three values, or leaving the age with two values (eg 5/4). For the preparation of age-length keys, those *illicia* with doubts between two ages, were assigned as belonging to the more confident age (the value firstly located, eg. 5 for age 5/4);

AQ3. *Illicia* practically unreadable or very difficult to age, with doubts among three or more possible ages. Those *illicia* were excluded from further analysis.

3.5.2 DAILY AGE

To test the quality control of daily age estimates, internal (reading procedure) practices are developed by IEO. Each *lapillus* was read at least two times until a consistent increment count was obtained. When error in reading precision was more than 10%, a third reading was taken. If the discrepancy persisted, the *lapillus* was discarded.

4 BLACK ANGLERFISH (LOPHIUS BUDEGASSA)



Fig 4.1. Dorsal and ventral view of *L. budegassa*, showing the black peritoneum.

Species identification

By Bauchot and Pras (1987):

The main morphological characteristics of this species are similar to those of its congener *L. piscatorius* (section 3), differing morphologically from it mainly by the black peritoneum (Fig. 4.1) and the second dorsal fin with 9-10 rays.

***Illicium* morphology**

The morphological characteristics of the *illicium* of this species are very similar to those of *L. piscatorius* (section 3).

4.1 SAMPLING PROGRAM FOR AGE ESTIMATION

4.1.1 ANNUAL AGE

Routine samplings for age determination of *L. budegassa* began to be performed in IEO since the mid 90s, from both, commercial catches and research surveys. The number of *illicia* collected in 2012 is shown in Table 4.1.1.1.

Table 4.1.1.1. Number of *L. budegassa illicia* collected from the commercial fleet and research surveys in 2012.

Stock	Commercial fleet	Research surveys	Total
ICES Div. VIIb-k, VIIIabd	404	35	439
ICES Div. VIIIc, IXa	446	98	544
Total	850	133	983

4.1.2 DAILY AGE

Lapilli otoliths from juvenile *L. budegassa* caught in the routine research surveys in 2010 and 2012, were collected for daily growth studies and otolith microstructure analysis (Table 4.1.2.1).

Table 4.1.2.1 Number of *L. budegassa lapilli* otoliths collected for daily growth studies from research surveys during 2010 and 2012 in ICES Div. VIIIc, IXa.

Year	Research surveys
2010	37
2012	21
Total	58

4.2 ILLICIA/OTOLITH EXTRACTION AND STORAGE

The methods for obtaining the *illicium* (for annual age) and the otoliths (for daily age) are the same as those used for *L. piscatorius* (section 3).

4.3 ILLICIA/OTOLITH PREPARATION AND AGE ESTIMATION METHOD

4.3.1 ANNUAL AGE

The traditional methodology of *illicia* mounting in resin plates was the same as that of *L. piscatorius* (section 3). It was originally described by Dupouy *et al.* (1986) and, after several European age estimation workshops of anglerfish (Anon, 1997; Anon, 1999; Landa *et al.*, 2002), it was standardized and was included in an age estimation guide for anglerfish (Duarte *et al.*, 2002). That *illicia* methodology was used in most of the growth studies using *illicia* (Duarte *et al.*, 1997; Quincoces *et al.*, 1998b; Landa *et al.*, 2001).

The age of *L. budegassa* is not routinely estimated by the IEO age readers, although the *illicia* are being collected in the routinely samplings (section 4.1.1). The aforementioned methodology of *illicia* preparation is available, but an age estimation criterion validated / corroborated is not currently available for this species. There is not enough knowledge on the real annual growth pattern of *L. budegassa*, but some solid research steps are being taken to estimate it (section 4.4.1). Thus, the recent advances in its otolith microstructure analysis (La Mesa and De Rossi, 2008; Hernández *et al.*, 2012) can help to locate the first annulus more precisely. Further research using several methods, as length frequency analysis, tagging-recapture studies, etc, is also necessary to obtain a better knowledge about the true growth pattern of *L. budegassa*.

4.3.2 DAILY AGE

The otoliths preparation and age estimation method for daily growth studies is the same as that used for *L. piscatorius* (section 3).

4.4 AGE ACCURACY, VALIDATION AND CORROBORATION

4.4.1 ANNUAL AGE

The age estimation from *illicia* of a decadal time-series was performed for the southern stock (Iberian Atlantic) assessment of *L. budegassa*, using the internationally standardized age estimation criterion of Duarte *et al.* (2002). A catch-at-age by year matrix was built, but inconsistencies in cohort tracking were found (Azevedo *et al.*, 2008). Since then no age-structured model has been used for the assessment of both northern and southern stocks of the European Atlantic southern shelf of *L. budegassa* (ICES, 2011a). A production model (ASPIC, Prager, 1994) has been used for the assessment as an alternative to the age-structured models.

The daily growth studies of La Mesa and De Rossi (2008) and Hernández *et al.* (2012) showed a faster growth pattern in the early stages of *L. budegassa* in Mediterranean and Atlantic waters respectively. Both studies also showed that juveniles at least up to 20 cm collected in September or October were born in the same year and, therefore, belong to age class 0. As the traditional annual age estimation criterion based on *illicia* that was used in the assessment of Atlantic stocks was being underestimated, those results may be also basic to establish a new and corroborated age estimation criterion using hard parts, in a similar way to that occurred in *L. piscatorius*.

4.4.2 DAILY AGE

The daily periodicity of micro-increment deposition has not been validated in early life stages of *L. budegassa* by any of the validation techniques available for daily age (Campana 2001). There is only one report on daily growth increment counts for this species performed on *lapilli* otoliths of juveniles captured in the Adriatic Sea (Mediterranean waters) (La Mesa and De Rossi, 2008).

4.5 AGE PRECISION, VERIFICATION AND QUALITY CONTROL

4.5.1 ANNUAL AGE

As in its congener *L. piscatorius*, the growth pattern of *L. budegassa* is also easier to distinguish in the *illicium* (Anon, 1991) and it has become the standard structure for age estimation in most European countries.

The age estimation criterion on both *Lophius* species used by IEO readers was externally verified in international *illicia* and otoliths exchanges and workshops (Anon, 1997; Anon, 1999; Landa *et al.*, 2002; Duarte *et al.*, 2005). The current age readers of IEO participated in the recent exchanges and workshops, showing good values of agreement, precision and relative accuracy for *illicia* (Duarte *et al.*, 2005). However, improving the precision in the absence of accuracy cannot, under any account, guarantee data quality (de Pontual *et al.*, 2006). And the age criteria for this species was internationally standardized and verified age of readers until 2007, did not mean that this criterion was not biased, as previously explained.

In addition to the estimated age, the quality (or credibility) of each age estimation is also assigned according to the "3 point grading system" recommended by WKNARC-2 (ICES, 2013), distinguishing 3 possible results of age quality (AQ) as explained for *L. piscatorius* (section 3).

4.5.2 DAILY AGE

To test the quality control of daily age estimates, the same internal (reading procedure) practices as those used for *L. piscatorius* (section 3) are applied in *L. budegassa*.

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